Detecting PCR Pathogens in Real Time

Brucellosis in such wildlife species as bison and elk is endemic and is a concern for the cattle industry. INL researchers are developing a blood test to detect active *Brucella* infection in bison, other wildlife, and cattle. The detection of pathogens has traditionally been accomplished using a polymerase chain reaction (PCR) followed by electro-phoretic gel separation of amplified target DNA. The approach typically requires about 3 hours for assay results. Our development of real-time PCR allows the amplification process to be monitored during each cycle of the PCR process, rather than following completion. This decreases the time required for detection to approximately 30 minutes and also enables quantification of starting target DNA. Probes and melt analysis of products can be used to increase specificity and determine the purity of a PCR product. INL's acquired field-portable real-time PCR instrument, called *RAPID*, for Ruggedized Advanced Pathogen Identification Device, is used to optimize assays to detect *Brucella*. INL is also developing and testing reliable primer sets for strain identification. These tools will greatly aid in the study to determine the true potential for interspecies transfer of *Brucella* in the environment.

Results

Yellowstone National Park has been the focus for INL's field tests, given the large number of bison and elk within its boundaries.

Application of this new equipment and associated techniques allows INL to determine on-site the infection of bison and elk. The information will aid in decisions regarding the need for destruction of animals. Furthermore, real-time PCR will allow the longstanding question of transfer between species to be addressed. In addition, a multi-plex PCR/multiple probes assay holds the promise of allowing simultaneous detection and discrimination of several organisms in a sample.

In general, real-time PCR potentially applies to forensics and national security-related needs. Available field technology to meet law enforcement and international needs at border

check points will aid in controlling the spread of pathogens and other undesirable materials.

Continued on next page

Science

INL

Idaho National Laboratory

Continued from front

For more information

Technical Contact

Frank Roberto, Ph.D.
(208) 526-1096
Francisco.Roberto@inl.gov

Deborah Newby, Ph.D.
(208) 526-7779
Deborah.Newby@inl.gov

Management Contact

Don Maiers
Manager,
Biological Sciences
(208) 526-6991
Donald.Maiers@inl.gov

INL is a U.S. Department of Energy national laboratory operated by Battelle Energy Alliance

Selected Publications/ Presentations

D.T. Newby and F.F. Roberto, "Real-time PCR assay for field diagnosis of *Brucella abortus* in wildlife populations in Yellowstone National Park." Brucellosis 2003 International Research Conference, University of Navarra, Pamploma, Spain, September 15-17, 2003.

D.T. Newby, T.L. Hadfield and F.F. Roberto, 2003 "Real-time PCR detection of *Brucella abortus*: a comparative study of SYBR Green I, 5'-exonuclease, and hybridization probe assays." Appl. Environ. Microbiol. 69:4753-4759.

D.T. Newby and F.F. Roberto, "Rapid detection of pathogens: DNA diagnosis for *Brucella*." 58th Northwest Regional Meeting of the American Chemical Society, Montana State University, Bozeman, MT, June 12-14, 2003.

F.F. Roberto, and D.T. Newby, "Application of DNA diagnostics for *Brucella* to recent outbreaks in the Greater Yellowstone Area." ASM Biodefense Meeting, Baltimore, MA, March 9-12, 2003.

D.T. Newby, H.G. Silverman, T. Hadfield and F.F. Roberto, "Real-time PCR Detection of *Brucella abortus*: a Comparative Study of SYBR Green I, Taqman, and Hybridization Probe Assays," American Society for Microbiology, 102nd General Meeting, Salt Lake City, UT, May 2002, Poster Q-381.

F. F. Roberto and D. T. Newby, "DNA Technology—An Introduction to Real-time PCR," Hispanic Youth Symposium (Scientific/ Engineering Workshop), Sun Valley, Idaho, April 2001.

D. T. Newby and F.F. Roberto, "Application of the Ruggedized Advanced Pathogen Identification Device (RAPID) for Brucella detection," INEEL Biotechnology Seminar Series, July 2001.

F. F. Roberto, H. G. Silverman, M. Tsang, R. Rodriguez, "A PCR Diagnostic System for Brucella in Wildlife Species," USGS Bison Research Initiative, 1998–2000, Yellowstone National Park, Bozeman, Montana.